Mulvihill-Smith Progeria-Like Syndrome: A Further Report With Delineation of Phenotype, Immunologic Deficits, and Novel Observation of Fibroblast Abnormalities

Deepthi C. de Silva,[1*] Denys N. Wheatley,[2] Richard Herriot,[4] Thelma Brown,[1] David A.J. Stevenson,[1] Peter Helms,[3] and John C.S. Dean[1]

[1]Department of Clinical Genetics, Medical School, University of Aberdeen, Foresterhill, Aberdeen, Scotland
[2]Department of Pathology, Medical School, University of Aberdeen, Foresterhill, Aberdeen, Scotland
[3]Department of Child Health, Medical School, University of Aberdeen, Foresterhill, Aberdeen, Scotland
[4]Department of Immunology, Aberdeen Royal Hospitals NHS Trust, Aberdeen, Scotland

We report the seventh case of Mulvihill-Smith progeria-like syndrome in a 5-year-old boy with a thin, pinched face, failure to thrive, and cutaneous pigmented nevi. The patient's motor and intellectual development were normal. His immune function tests demonstrate evidence of lymphopenia with no selective loss of a major subpopulation, low immunoglobulin (Ig)G2 and IgG4 subclasses, and an absent in vitro proliferative response to pokeweed mitogen. Chromosomal mitomycin and radiation sensitivity were normal. The skin fibroblast growth in culture was slow, and the fibroblasts appeared morphologically different from normal controls in their size and large number of inclusions. In addition, primary cilia, which normally issue from the centrosome, were absent—a new finding in fibroblasts in this disorder. It remains to be seen if the relative absence of centrosomal cilia in cultured fibroblasts in early passages is a consistent finding in this progeria syndrome. Am. J. Med. Genet. 69:56–64, 1997.

KEY WORDS: Mulvihill-Smith syndrome; progeria; immune deficiency; centrosomal primary cilia; pigmented nevi

INTRODUCTION

The Mulvihill-Smith progeria-like syndrome (McKusick 176690) is a rare disorder, with six reported cases in the medical literature. It is characterised by a "pinched" facial appearance with hypoplasia of the lower half of the face. Reduced facial subcutaneous fat causes a prematurely aged appearance, but there is a relatively normal subcutaneous fat distribution in the trunk. Other anomalies include multiple pigmented nevi of the skin, high-pitched voice, growth failure (which is prenatal in onset), and a progressive immune deficiency of varying degrees of severity. Mental retardation, genital abnormalities, hearing loss, diabetes, hepatic dysfunction, and dental anomalies have also been reported.

CLINICAL REPORT

The propositus was the child of a healthy, unrelated Scottish couple, the father aged 42 years and the mother aged 33 years. There is no history of relative infertility in the parents, although the propositus is an only child. He was born by spontaneous vaginal delivery at 38 weeks of gestation. Oligohydramnios and poor fetal growth had complicated the pregnancy. His mother denied any infections during pregnancy, and there was no history of exposure to drugs or alcohol. At birth, he weighed 2,600 g (10–25th centile), the head circumference (OFC) was 32.5 cm (10–25th centile), and the crown heel length (CHL) 48 cm (10–25th centile). Undescended testes were noted, and orchidopexies were performed at 2.5 years.

His postnatal growth was slow, and he was referred at 3 years for assessment of failure to thrive. On examination, he had a pinched nose, loss of facial subcutaneous fat (particularly in the lower half of the face), micrognathia, and sunken eyes (Fig. 1a,b). The subcutaneous fat distribution in the trunk and limbs appeared to be normal (Fig. 1c). Multiple pigmented nevi (Fig. 1d) were found on his trunk, especially on the back. Apart from mild pectus carinatum, no other skeletal abnormalities were noted. Cardiovascular examination

*Correspondence to: Dr. Deepthi de Silva, Department of Clinical Genetics, Medical School, Foresterhill, Aberdeen, AB9 2ZD, Scotland.

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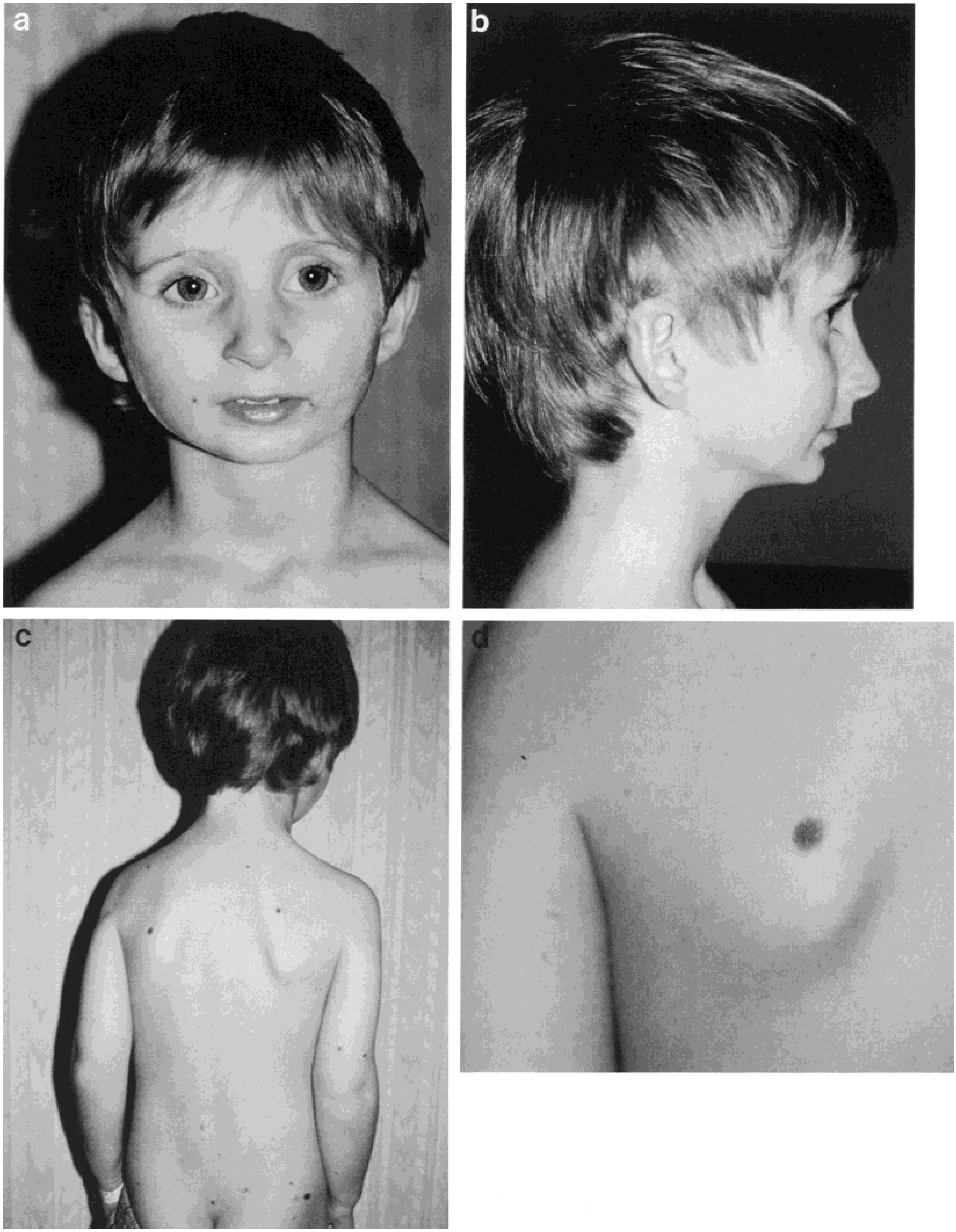

Fig. 1. **a, b:** Propositus aged 4.5 years. **c:** Normal fat distribution of trunk and pigmented nevi. **d:** Pigmented nevus.

demonstrated a soft systolic murmur. There was no clinical evidence of hepatosplenomegaly or signs of chronic liver disease. Neurologic examination was normal, as was his hearing on clinical assessment. He was far sighted but has not required visual correction. At 3 years and 9 months, his weight was 13.98 kg (10th centile), OFC was 48.9 cm (10th centile), height was 98.5 cm (50th centile), total hand length was 11.3 cm (25th centile), and middle finger was 4.9 cm (25–50th centile). His teeth and hair were normal. He had no history of severe infections, but had verrucae on his feet, a history of an episode of gastroenteritis, and two episodes of rectal bleeding (for which no cause was identified). At 5 years, he remains developmentally normal and his growth parameters remain within the normal range, with height at 105.6 cm (25th centile) and weight at 16 kg (10th centile). His pigmented skin lesions are increasing in number. He has a high-pitched voice but no other abnormalities of articulation.

Results of Investigations

The patient was found to have lymphopenia, with a lymphocyte count of 0.68×10^9 (normal, 1.6–7.0). Lymphocyte subset analysis confirmed lymphopenia with low numbers of both main T-cell subsets (but especially CD4+ T cells), B cells (CD19+), and NK cells (CD16+, CD56+). There was no absolute loss of any major subpopulation. Mitogen lymphocyte stimulation assay demonstrated an absent response to pokeweed mitogen but normal response to PHA and Con-A. Immunoglobulin (Ig) assay was within the normal range for a child of this age: total IgG, 5.6 g/l (normal, 4.9–16.1); IgA, 0.5 g/l (normal, 0.4–2.0); IgM, 0.6 g/l (normal, 0.5–2.0); and IgE, 22.8 IU/ml (normal, 2–52). Specific IgG subclass analysis showed a low IgG2 and IgG4 at 67.6 mg/dL (normal, 70–450) and 2.6 mg/dL (normal, 10–80), respectively. Salivary IgA was present. C3 (125 mg/dL; normal, 88–201) and C4 (29.7 mg/dL; normal, 16–47) levels were normal, as were functional complement assays (CH50 and alternate pathway hemolytic activity). Results of routine biochemistry tests including urea, electrolytes, and liver function tests were normal.

G-banded lymphocyte chromosome analysis at metaphase showed a normal, 46 XY karyotype (resolution of 550 bands per haploid karyotype). There was no evidence of increased sensitivity to mitomycin C. Lymphocytes exposed to 0.5 and 1.0 Gy radiation demonstrated no altered sensitivity compared with a control. In addition, there was no evidence of spontaneous chromosome breaks found in 50 metaphases examined.

Skin fibroblasts demonstrated no evidence of sensitivity to ultraviolet light at 2, 5, 10, and 20 Jm^{-2} compared with an age-matched control, although they grew more slowly. However, their morphology in culture was distinct from that of the normal skin fibroblasts (Fig. 2a), the cells being kite-like in shape and more thinly spread over the substratum (Fig. 2b). The cells initially obtained grew to confluence in this highly spread condition and were subcultured twice onto glass coverslips. We examined them on both these occasions by ID5 staining for detyrosinated α-tubulin, as previously described [Wheatley et al., 1994, Strugnell et al., 1996]. The cells seemed to lack primary cilia, although their centrosome staining was normal (Fig. 2d). This contrasts with normal fibroblasts (from young boy's foreskin) that invariably possess primary cilia (Table I; Fig. 2c). The cells were re-examined several months later after regrowth from storage, after which they grew rapidly and formed a more or less confluent monolayer very quickly. They had taken up an appearance much closer to that seen in control fibroblasts (Fig. 2a) and now showed a cilium incidence approaching 50%. Although this was significantly lower than the controls, it was in stark contrast to the earlier data (Table I). For comparison, and in the absence of fibroblasts from another Mulvihill-Smith syndrome patient, we obtained a culture of immortalised fibroblasts from a patient with Werner syndrome. These exhibited a similar tendency, showing little or no evidence of cilia until they had gone through 13–14 passages in culture, at which time they reverted to a situation similar to controls but with lower cilium incidence than seen in our patient's fibroblasts (Table I). In these cultures, centrioles and midbodies (noted for their strong staining with ID5) were clearly evident. These features provide internal controls, which indicate that if primary cilia were present, they would have been positive (as in the control fibroblasts).

DISCUSSION

Since the first report [Mulvihill and Smith, 1975] of a 17-year-old boy with prenatal short stature, pinched face, premature ageing, multiple pigmented nevi, mental retardation, photosensitivity, thin skin, sparse hair, and oligodontia, five other patients [Shepherd, 1971 and Elliot, 1975; Wong et al., 1979; Baraitser et al., 1988; Ohashi et al., 1993; Bartsch et al., 1994] with Mulvihill-Smith syndrome have been identified. Table II provides a summary of the phenotype in the present case and the six previous cases in the literature.

Our patient has the facial features and pigmented nevi described in all the previous case reports. The high-pitched voice noted in this case and in three others [Shepherd, 1971; Mulvihill and Smith, 1975; Bartsch et al., 1994] may reflect the abnormal facial structures. Although he has no evidence of severe immune deficiency clinically, the lymphopenia with reduction of T-cell and B-cell numbers is similar to those previously described [Bartsch et al., 1994]; the in vitro functional immune deficits appear more limited, and the pattern of immunoglobulin abnormalities in our case is restricted to low IgG2 and IgG4 subclasses. Urogenital abnormalities in the other cases include cryptorchidism, hypospadias, undescended testes, and amenorrhoea [Ohashi et al., 1993]. The undescended testes in the present case probably represent a manifestation of this syndrome.

Our patient differs from most of the other reported patients with respect to the severity of growth retardation. Our patient was above the 10th centile for weight, height, and OFC at birth and at 5 years and remains at the 25th centile for height and the 10th centile for weight. Moderate to severe mental retardation has been reported in some of the previous patients but is not present in this patient, although Bartsch et al. [1994] reported that their patient deteriorated intellec-

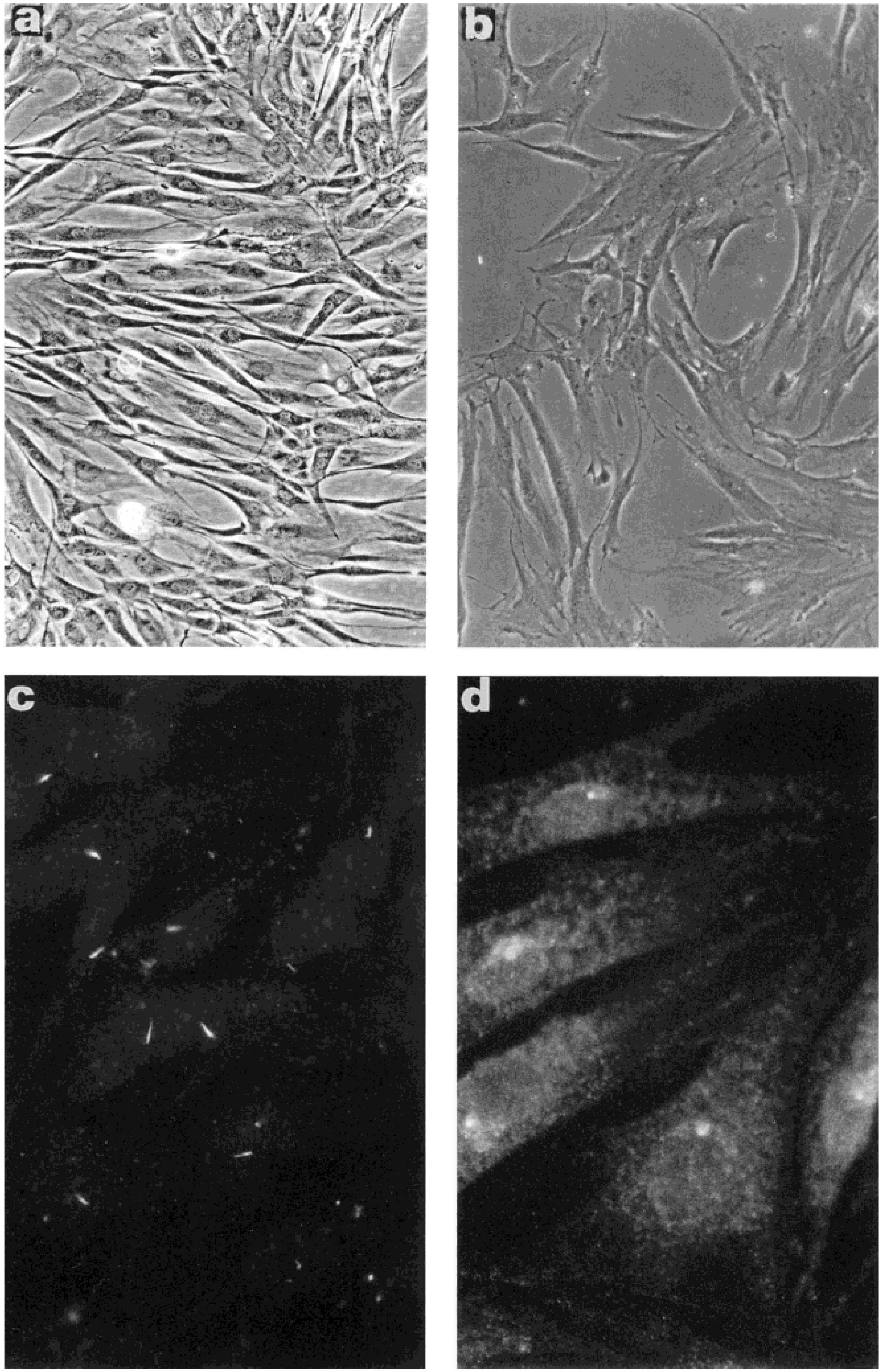

Fig. 2. **a:** Normal human foreskin fibroblasts growing in vitro. First subculture following primary culturing. Phase contrast ×350. **b:** Patient's fibroblasts grown under identical conditions. ×350. **c:** ID5 immunofluorescence micrograph of normal human diploid fibroblasts as in a, showing the high level of primary ciliation of cells, ×750. **d:** ID5 immunofluorescence micrograph of patient's fibroblasts showing centrosomes but no evidence of ciliation, ×1,400.

TABLE I. Details of Cultures Assessed for Presence of Primary Cilia

Case	Passage number	% Confluence	Ciliated cells	Total cells counted	% ciliated ± 1SD counted
Mulvihill-Smith syndrome	3	40–50	0	345[a]	0
Mulvihill-Smith syndrome	5	60–65	1(?)	381[a]	<1
Mulvihill-Smith syndrome	6	95	83	186	44.6
			130	270	48.1
			56	124	45.2
			269	580	46.7 ± 1.7*
Werner syndrome[b]	11	85	0	314[a]	0
Werner syndrome	12	90	2(?)	350[a]	<1
Werner syndrome	14	95	37	200	18.5
			27	180	15.0
			45	190	23.7
			109	570	19.1 ± 4.4*
Control		50	231	357[a]	65.8 ± 3.7
Control		95	332	355[a]	93.5 ± 4.0

* $P < 0.05$ in both cases compared with control fibroblasts.
[a] Three separate cultures and not less than 100 cell assessed in each case, with data pooled for brevity. Assessment showed no significant variations between two observers.
[b] See Kill et al. [1994] for details. The cells used here are referred to as WS/strain BJ846.
Controls were consistent and analyzed at the same time.
Full details of culture methods, media, and ID5 staining for detyrosinated tubulin can be found in Wheatley et al. [1994] and Strugnell et al. [1996].

tually with age. Our patient does not have the brachydactyly reported in four previous patients. The normal appearance of the present patient's hair and teeth also contrasts with some of the previous reports. Five previously reported patients have also had sensorineural deafness, which was not present in our patient or the one reported by Bartsch et al. [1994]. The milder phenotype of our patient may reflect the early stages of the Mulvihill-Smith syndrome, with the prospect of further deterioration in growth centiles and intelligence in the future; however, it may equally be that he has a milder form of this syndrome that may be underrecognized due to the absence or mildness of the cutaneous features.

The multisystem involvement in this progeria-like syndrome, and the fact that one of the patients was the child of a consanguineous couple, has led to the suggestion that this may be an autosomal recessive disease. However, no recurrence within a family has been reported, and the advanced paternal age in this and the case reported by Mulvihill and Smith [1975] would also be compatible with a new dominant mutation.

The Mulvihill-Smith syndrome has similarities to other progeria-like disorders with onset in the first decade (Table III). DNA repair defects have been identified in Cockayne syndrome (CS), involving the excision repair or the post-ultraviolet (UV) damage replication pathway. Ohashi et al. [1993] demonstrated altered X-ray sensitivity in their patient's fibroblasts compared with a healthy control, although they accept that the result may have been affected by the prolonged doubling time of the patient's fibroblasts. Mulvihill and Smith's patient is reported as having UV sensitivity, although similar fibroblast sensitivity was reported by the authors in one of their controls, a diabetic patient. In our much younger patient, there was no demonstrable evidence of UV or X-ray sensitivity. Therefore, these findings are not consistent in the Mulvihill-Smith syndrome and do not provide evidence that defects in the DNA excision repair pathway are implicated in the pathogenesis of this disorder.

Slow growth of cultured fibroblasts in Mulvihill-Smith syndrome was documented by Ohashi et al., [1993] and Mulvihill and Smith [1975]. Our patient's fibroblasts were also slow growing; in addition, they had an unusual, kite-like appearance (Fig. 2b) more usually seen in highly spread cells that take a long time to traverse their generation cycle. There was a much higher incidence of cytoplasmic inclusions and stress fibres. Their flat extensions tended to lie over one another as they grew toward a confluent monolayer. In the flow cytometer, their nuclear size and cycle distribution were not significantly different from that of controls. There was little or no evidence of ciliation of their centrosomes in the early cultures that were examined, whereas there was a high level of cilium expression in normal fibroblasts [Wheatley et al., 1994] from primary cultures right through to their Hayflick limit (the maximum number of generations achieved by continuous subculturing). Fibroblasts cultured from skin disorders such as keloid and hypertrophied scars tend to grow slowly, contain more than the normal number of inclusions, but regularly express normal amounts of cilia, although this is not as high as normal fibroblasts [Strugnell et al., 1996]. When subcultured, the patient's fibroblasts reverted to a morphology closer to that of normal fibroblasts, growing faster and having fewer inclusions. In further subcultures from the original stock, cilium expression was observed (Table I, line 3), but at

TABLE II. Mulvihill-Smith Syndrome: Summary of Previous and Present Case Reports*

	Mulvihill and Smith [1975] (male 17 yr)	Shepherd [1971] and Elliot [1975] (male 3 yr/4.5 yr)	Wong et al. [1979] (female 14 yr)	Baraitser et al. [1988] (male 7 yr)	Ohashi et al. [1993] (female 30 yr)	Bartsch et al. [1994] (male 20 yr)	Present study (male 4 yr)
Family history							
Parents' age (mother, father)	40 yr, 46 yr	21 yr, 21 yr	20 yr, 26 yr	34 yr, 36 yr	29 yr, 27 yr	29 yr, 33 yr	33 yr, 42 yr
Consanguinity	No	No	No	No	Yes	No	No
Family history	Maternal diabetes, pancreatitis	FH of diabetes				Mother tall	
Birth and development							
Pregnancy						Hyperemesis, poor growth	Oligohydramnios, poor growth
Gestation	NR	40	40	39	39	40	38
Birth weight	1,800 g	1, 890 g	1,800 g	1,880 g	2,700 g	3,340 g	2,600 g
CHL	NR	NR	NR	NR	NR	52 cm	48 cm
Present height centile	<3rd	Short	NR	NR	−1.9 SD	−2.6 SD	50th
Present weight centile	<3rd	<3rd	Low	<3rd	NR	−11%	<3rd
OFC (centile)	<3rd	Microcephaly	Microcephaly	<3rd	−2.6 SD	−3.1 SD	3–10th
Intellectual development	IQ 80	IQ <50	Normal	Mild ↓	Severe ↓	Mild ↓	Normal
Syndrome manifestations							
Speech	High pitch	NR	NR	High pitch	None	High pitch	High pitch
Sunken eyes	+	+	NR	NR	+	+	+
Lower facial hypoplasia	+	+	+	+	+	+	+
Hypertelorism	NR	Blepharo-phimosis	+	−	+	+	−
Pigmented nevi	At birth	11 mo	7 yr	+	+	1 yr	At birth
Facial fat reduced	+	NR	+	+	+	+	+
Alopecia	Sparse	Sparse	NR	NR	NR	Hirsute	Normal
Thin skin	+/Dry	Scalp veins	NR	NR	NR	Dry	Normal

(continued)

TABLE II. Mulvihill-Smith Syndrome: Summary of Previous and Present Case Reports* *(continued)*

	Mulvihill and Smith [1975] (male 17 yr)	Shepherd [1971] and Elliot [1975] (male 3 yr/4.5 yr)	Wong et al. [1979] (female 14 yr)	Baraitser et al. [1988] (male 7 yr)	Ohashi et al. [1993] (female 30 yr)	Bartsch et al. [1994] (male 20 yr)	Present study (male 4 yr)
Syndrome manifestations							
Wrinkled skin	NR	NR	NR	NR	+	NR	Normal
Oligodontia	+	Conical canines	+	+	NR	+	No
Deafness (sensorineural)	+	+	+	+	3 yr	?No	No
Hands	3–5 MCP short	Brachymeso-phalangy, clinodactyly 5th	NR	NR	Distal brachydactyly	Distal brachydactyly	Normal
Bone vs. chronologic age	?Same	Delay	NR	Advanced	NR	NR	NR
Genitourinary anomalies	Hypospadias	Cryptorchidism, hypospadias	Tanner 3/4, amenorrhoea,	Hypospadias	Early menopause	Tanner 2/4	Orchidopexy
Immune dysfunction	Caries, chronic cough	Periodontitis, otitis media	Periodontitis	NR	Respiratory infection recurrence, otitis media, warts	Conjunctivitis, rhinitis	Diarrhea, rectal bleeding, verrucae
Other	Diabetes 14 yr	Vomiting, FTT	Hepatomegaly, hyper-lipidaemia	Hepatomegaly, abnormal LFT		Depression, hyperlipidaemia	Systolic murmur
Investigations							
T cells	NR	NR	NR	NR	CD 2/3/4/8/20 Normal Mito. res ↓	T cells inc CD 3/4 ↓ Mito. res ↓	Low T cells ↓ CD4 Mito. res ↓
B cells	IgA borderline	NR	NR	↓ IgG	↓ IgG/A	IgG/A ↓ B cells ↑	IgG2 and IgG4 ↓
X-ray sensitivity	NR	NR	NR	NR	Slightly ↑	NR	Normal
UV sensitivity	66–77%	NR	NR	NR	NR	NR	Normal
Fibroblast growth	Delayed	NR	NR	NR	Delayed	NR	Delayed

*FH, family history; CHL, crown heel length; NR, not recorded; SD, standard deviation; OFC, head circumference; Mito, Mitomycin; MCP, metacarpophalanges; FTT, failure to thrive; LFT, liver function tests; Ig, immunoglobulin; UV, ultraviolet.

TABLE III. Progeria Syndromes With Onset in the First Decade Sharing Similarities With Mulvihill-Smith Syndrome (Based on Gorlin et al. [1990])*

	Hutchison-Gilford progeria syndrome	Wiedeman-Rautenstrauch syndrome	Rothmund-Thompson syndrome	Cockayne syndrome	Mulvihill-Smith syndrome
Minor anomalies	Reduced facial fat, small face, micrognathia Loss of scalp, eyebrow and eyelash hair	Aged appearance, frontal and parietal bossing, beaked nose	Large head, frontal bossing	Loss of facial fat, sunken eyes, microcephaly	Loss of facial fat, microcephaly, hypoplasia of lower face
Onset	2nd year of life	Prenatal	1st year of life	Prenatal or neonatal	?Prenatal
Skin manifestations	Atrophy and scarring	Sparse scalp hair	Pigmentation, atrophy and telangectasia, sparse hair, warty light sensitivity keratosis, verrucous changes	Dermatitis, scarring, and pigmentation, UV sensitivity	Pigmented nevi
Growth	Severe retardation	Retarded	Proportionate short stature, hypertension	Disproportionate short stature, hypertension	?Retarded
Cardiovascular	Hypertension, atherosclerosis				
Genitourinary			Micropenis, hypogonadism	Undescended testes	Hypospadias, cryptorchidism, early menopause
Development	Normal IQ	Severe motor and mental delay, progressive deterioration	Normal	Progressive neurodegeneration	Delayed development, ?deterioration
Musculoskeletal	Contractures, horse riding stance, osteoporosis, osteolysis of fingers		Contractures, absent thumbs, abnormal ulnae, radii	Osteoporosis, horse riding stance	
Other	High-pitched voice	CNS demyelination, respiratory infections	Cataract, risk of malignancy	Ataxia, microcephaly, salt and pepper retina, cataracts, no malignancies	High-pitched voice, immune deficiency
UV or radiation sensitivity	Nil			Increased UV and mitomycin sensitivity	?Normal
Inheritance	Not known	Autosomal recessive	Autosomal recessive	Autosomal recessive	Not known

*UV, ultraviolet; CNS, central nervous system.

a significantly lower incidence than in control fibroblasts. We have also examined the fibroblasts of a cell line from a patient with Werner syndrome [Kill et al., 1994] in which early passages showed little or no evidence of primary cilia, but between the 12th and 14th passages, started to grow more rapidly and express primary cilia (Table I).

The function of the centrosomal cilia is not clear, but they probably have chemosensory and mechanosensory functions that relay information about their immediate environment to the centrosome. The mechanism regulating the expression of cilia is also unknown. Studying situations in which a clear distinction in their presence can be seen under different environmental and genetic influences may provide further information about the function and control of expression of centrosomal cilia. The distinctive differences in the fibroblasts from this patient suggests that similar studies are needed on patients with this and other progeria-like syndromes to identify if this is a consistent finding.

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